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A MIXED INFECTION OF *PASSIFLORA CAERULEA* L. WITH TWO VIRUSES

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Two viruses were isolated from the ornamental plant *Passiflora caerulea* L. with spotting, vein clearing and deformation of leaflets. One of the viruses from the mixed infection was cucumber mosaic virus. The other one was established to be an isolate of bean yellow mosaic virus (BYMV). The isolate of BYMV infected *Chenopodium quinoa* and *C. amaranticolor* systemically and induced wilting and dying in *Pisum sativum* cv Petit Provençal. The infection of *Phaseolus vulgaris* cv Perlička and *Vicia faba* was achieved with difficulty. Most of the cytological changes it provoked characterize BYMV infections, however, the lack of protein crystals in the nucleoli and the reaction of the test plants mentioned above indicate its difference from ordinary BYMV isolates.

To the best of our knowledge *P. caerulea* is a new natural host of BYMV.

Introduction

The climbing plant *P. caerulea* is an exotic ornamental species with fairly attractive flowers (Fig. 1. A). Two different viruses have been reported from this plant up to now. One of them is a carlavirus, *Passiflora* latent virus (Schnepf and Brandes 1961, Brandes and Wetter 1963, Bos and Rubio-Huertos 1971) which appears to be common in *P. caerulea*. The other one is cucumber mosaic virus (Teakle et al. 1963, Zschau 1964) wide-spread in general.

From the virus infected specimen of *P. caerulea*, in addition to cucumber mosaic virus (CMV) we have isolated a potyvirus which seems to be an isolate of bean yellow mosaic virus (BYMV). This paper presents the work determining the identity of these two viruses.

Material and Methods

Material

The infected *P. caerulea* from which both viruses were obtained grew in the glasshouse of the University Botanic Garden, Zagreb. The plant showed prominent spotting, vein clearing and deformation of leaflets (Fig. 1. B). The symptoms were well defined especially early in the spring.

Methods

For isolation of the viruses the younger symptoms bearing leaves were homogenized in 0,06 M phosphate buffer pH 7,2. In order to lower the activity of inhibitors present a small quantity of charcoal was added to the inoculum which was rubbed on the leaves of *Chenopodium quinoa.* The potyvirus was separated from the mixture by using systemically infected *C. quinoa,* and CMV by passage through *Datura stramonium* which is not the host of the isolated potyvirus.

C. quinoa served as propagation and assay species. Host range studies were done with sap stabilized by phosphate buffer. Back inoculations were also made to *C. quionoa.*

To determine the stability of the virus in crude sap conventional procedures (Bos et al. 1960) using crude sap from systemically infected *C. quinoa* were applied.

Light-microscopical cellular inclusions were studied in living state in epidermal strips with hair cells of systemically infected *Nicotiana clevelandii* leaves.

Ultrathin sections were made from the pieces of systemically infected *C. quinoa* and *Vicia faba* leaves embedded in Araldite according to the procedure by Pleše and Wrischer (1978). To control the size of filamentous virus particles in dip preparations, crude sap from infected *C. quinoa* was mixed with 2% neutral sodium phosphotungstate and layered on formvar-coated grids.

Serological test with CMV was done by double diffusion method in 0,9% agar with 0,05% NaN_3 which had been prepared in distilled water. The inoculated leaves of *C. quinoa* were used as source of viral sap and the sap of the healthy plant as control. The serum used against Car strain of CMV from carnation (Lovisolo et al. 1968) was supplied by Dr E. Luisoni, Torino. It contained besides antibodies against the whole virus particles (titre 1 : 128) also the antibodies against protein subunits (titre 1 : 64) and antibodies to normal host proteins (titre 1 : 16). To avoid reaction with normal plant constituents the serum was diluted 1/16 with distilled water.

Serological detection of isolated potyvirus was performed by double diffusion in agar gel containing sodium dodecyl sulfate (SDS) according to Hunst and Tolin (1982). The tests were done with the serum against B25 strain of BYMV (Bos 1970a, Bos et al. 1974) supplied by Dr D. Z. Maat, Wageningen. The homologous titre of the serum was 1 : 256. The serum was used undiluted and diluted 1/4. Crude sap of inoculated leaves of *C. quinoa* covered with uncountable local lesions was centrifugated at low speed (4000 rpm/20 min) and used as antigen. The sap of healthy *C. quinoa* treated in the same way served as control.

Results

Finding of cucumber mosaic virus

The identity of isolated CMV was confirmed on the basis of the results of investigations as follows.

The host range and symptomatology of isolated virus corresponded to those characteristic of CMV (Francki et al. 1979). *C. quinoa, C. amaranticolor, Phaseolus vulgaris* and *Vigna sinensis* reacted with local lesions whose morphology was typical of CMV. *Cucumis sativus* cv Delicates, *Cucurbita maxima, C. pepo* (Fig. 1. C), *Datura stramonium, Nicotiana glutinosa, N. megalosiphon* and *N. tabacum* cv Samsun reacted systemically. The appearance of symptoms corresponded also to those provoked by many CMV isolates.

In the serological tests our isolate in crude sap reacted with the serum against Car strain of CMV by the apparition of straight precipitation lines (Fig. 1. D) specific for soluble virus protein, i.e. decomposed virus particles (Scott 1968). Such straight precipitation lines appeared because the infectious sap was not extracted by stabilizing buffer (Devergne et al. 1972, Štefanac et al. 1981).

Consequently, the investigations described have shown that one of the two viruses causing the mixed infection of *P. caerulea* is an isolate of CMV.

Finding of bean yellow mosaic virus

In addition to CMV a potyvirus was detected in a mixed infection of *P. caerulea.* In order to identify it the host range and symptomatology of the virus were studied closely at first.

The virus infected *C. quinoa* systemically causing chlorotic lesions in inoculated leaves followed by bright yellow spotting, leaf deformations and slight stunting of the whole plant (Fig. 2. A). *C. amaranticolor* developed chlorotic lesions in inoculated leaves and systemic symptoms in the form of large chlorotic spots often extending into the veins (Fig. 2. B). *N. clevelandii* showed faint systemic mottling and necrotic spotting (Fig. 2. F). In *Phaseolus vulgaris* cv Perlička it caused necrotic lesions and vein necrosis in inoculated leaves (Fig. 2. D) and then systemic yellowish mosaic (Fig. 2. E), and in *V. faba* systemic yellowish spotting (Fig. 2. C). However, difficulties were encountered in its transmission to both hosts. In *Pisum sativum* cv Petit Provençal the virus provoked wilting and dying of the plants. *C. murale* and *Gomphrena globosa* reacted locally with necrotic and chlorotic lesions, respectively.

The following plants were not susceptible: *Capsicum annuum, Cucumis sativus* cv Delicates, *D. stramonium, N. glutinosa, N. megalosiphon, N. tabacum* cv Samsun, *Petunia hybrida* and *V. sinensis.*

Crude sap of systemically infected leaves of *C. quinoa* lost infectivity at dilution between 10^{-3} and 10^{-4}, after heating at 50°—55° C for 10 min, or after storage at room temperature for 4 to 5 days.

The symptomatology of the virus and its behaviour in crude sap indicate unequivocally that it can be aligned with the isolates of BYMV (cf. Bos 1970b, Bos et al. 1974).

Light and elecronmicroscopical studies of infected cells also revealed the changes which characterize BYMV infections (see: Bos 1969, Kamei et al. 1969, Edwardson 1974, Christie and Edwardson

1877, Martelli and Russo 1977, Russo et al. 1979, 1981, Alper and Loebenstein 1981 etc.).

As observed in the light microscope, large granular cytoplasmic inclusion bodies often located next to the nuclei were characteristic of infected cells (Fig. 3. A, B). In addition enlarged nucleoli could be detected within the nuclei (Fig. 3. A, B), but they showed no crystalline bodies, i.e. protein crystals embedded (cf. Bos 1969, 1970b).

In ultrathin sections distinctive cytological alterations were noticed, although general cellular architecture and major organelles were apparently unaffected. The cytoplasm contained cylindrical inclusions, sometimes with radiating plates. Typical pinwheels were rare (Fig. 3. C, D), whereas, laminated aggregates prevailed (Fig. 3. D, 4. A),as it is usually with BYMV (Kamei et al. 1969, Christie and Edwardson 1977, Russo et al. 1979, 1981, Alper and Loebenstein 1981 etc.). Additionally, clusters of small electron dense crystalline bodies associated with endoplasmic reticulum lay in some places in the cytoplasm (Fig. 4.), but were never observed in the nucleolar mass in the nuclei. Ribosome-like particles were always attached to the surface of the electron opaque bodies (Fig. 4. C). The filamentous virus particles were detected sporadically. It is evident that all submicroscopical structures and alterations described, often gathered in certain areas of the cytoplasm, appear as granular inclusions under a light microscope.

Negatively stained dip preparations contained elongated flexuous particles measuring about 440—750 nm.

In serological tests the virus reacted with the serum to B25 strain of BYMV forming a weak but specific precipitation line the appearance of which was characteristic of degraded virions. No precipitation line was observed using the sap of healthy plant.

Discussion

Our finding of CMV in *P. caerulea* was not an unusual event because up to now CMV have been reported several times on *Passiflora* species. Besides its detection in *P. caerulea* (Teakle et al. 1963, Zschau 1964) it has also been found repeatedly in passionfruit *(P. edulis)* (Ishii and Pascual 1964, Taylor and Kimble 1964). That our isometric virus belongs to CMV and not to the closely related peanut stunt virus was confirmed on the basis of its ability to infect *Cucumis sativus, Cucurbita maxima* and *C. pepo* and to produce local infection in *Chenopodium quinoa.*

Of the four elongated viruses reported to infect genus *Passiflora,* three have been studied sufficiently well. One of them, *Passiflora* latent virus (Brandes and Wetter 1963) belongs to the carlavirus group (Matthews 1982). The other two, passionfruit woodiness virus (Taylor and Greber 1973) and passionfruit ringspot virus (De Wijs 1974) are potyviruses. Our finding of BYMV in *P. caerulea* increases the number of potviruses isolated from *Passiflora.*

The difficulty with which the described isolate of BYMV infects leguminous hosts *(Phaseolus vulgaris* and *Vicia faba)* and the lack of protein crystals in nucleoli may be an indication of its difference from typical BYMV isolates, as it is the case with gladiolus isolate (Kamei et al. 1969) or saffron isolate (Russo et al. 1979).

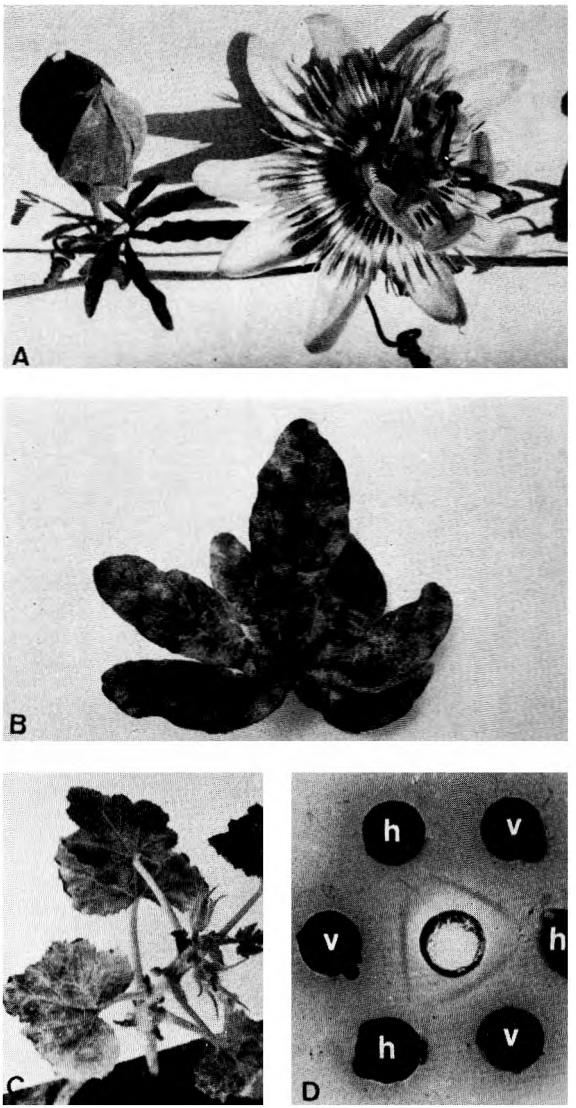

Fig. 1. **A** Flower of *Passiflora caerulea*. **B** Symptoms in *P. caerulea* from which both viruses were isolated. **C** Mosaic symptoms in *Cucurbita pepo* provoked by CMV isolate. **D** Immunodiffusion reaction with CMV isolate and the serum against CMV-Car (centre well); the peripheral wells contain the virus in crude sap (v) and, as control, the sap of healthy *Chenopodium quinoa* (h).

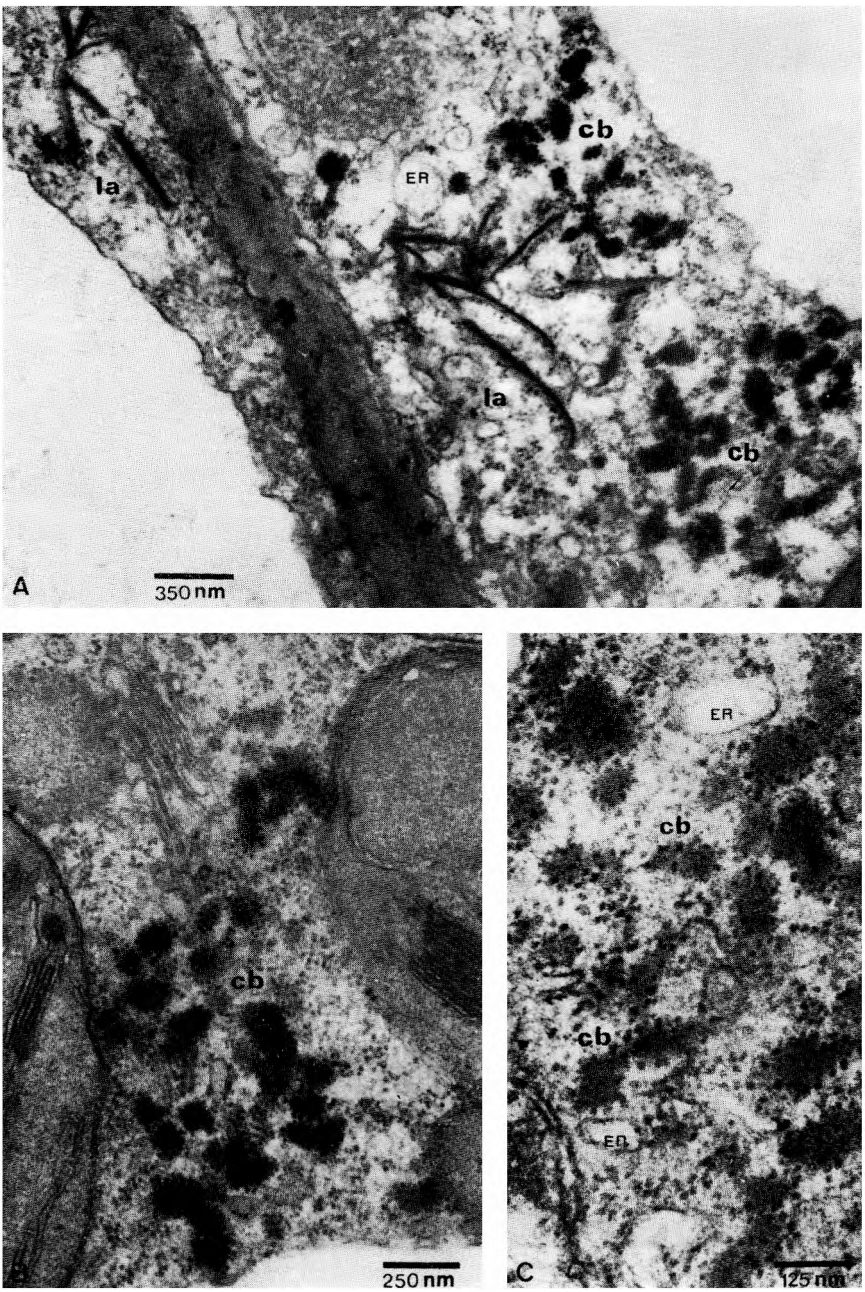

Fig. 4. As in Fig 3. **C, D,** but sections from *Vicia faba* showing laminated aggregates (la) in **A,** and electron dense crystalline bodies (cb) associated with endoplasmic reticulum (ER) in **A, B, C.**

As far as we know, *P. caerulea* is a new addition to the list of natural hosts of BYMV.

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SAŽETAK

SMJESNA INFEKCIJA U VRSTE *PASSIFLORA CAERULEA* L. DVAMA VIRUSIMA

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Iz ukrasne biljke *Passiflora caerulea* L. sa siptomima pjegavosti, prosvjetljavanja nerava i deformacije lisaka izdvojena su dva virusa. Jedan od virusa smjesne infekcije bio je virus mozaika krastavca (cucumber mosaic virus). Za drugi je virus utvrđeno da predstavlja izolat virusa žutog mozaika graha (bean yellow mosaic virus, BYMV).

Na vrstama *Chenopodium quinoa* i *C. amaranticolor* izolat BYMV izazivao je sistemičnu infekciju, a na vrsti *Pisum sativum* »Niski Provansalac« venuće i uginuće zaraženih biljaka. Vrste *Phaseolus vulgaris* »Perlička« i *Vicia faba* izolat je teško zaražavao. Većina citoloških promjena koje je izolat uzrokovao karakteristična je za infekciju BYMV; međutim, izostanak proteinskih kristala u jezgricama te reakcija gore navedenih pokusnih biljaka ukazuju da se izolat iz pasiflore razlikuje od većine izolata BYMV.

Koliko nam je poznato, *P. caerulea* je novi prirodni domaćin BYMV.

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